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Isolation of an Arsenate-Respiring Bacterium from a Redox Front in an Arsenic-Polluted Aquifer in West Bengal, Bengal Basin

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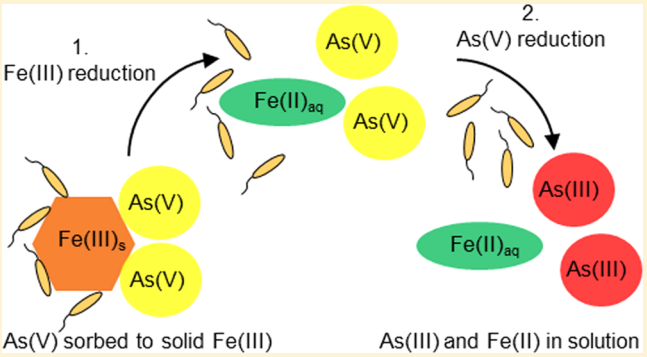
Supporting Information

ABSTRACT: Natural pollution of groundwater by arsenic adversely affects the health of tens of millions of people worldwide, with the deltaic aquifers of SE Asia being particularly polluted. The pollution is caused primarily by, or as a side reaction of, the microbial reduction of sedimentary Fe(III)-oxyhydroxides, but the organism(s) responsible for As release have not been isolated. Here we report the first isolation of a dissimilatory arsenate reducer from sediments of the Bengal Basin in West Bengal. The bacterium, here designated WB3, respires soluble arsenate and couples its reduction to the oxidation of acetate; WB3 is therefore implicated in the process of arsenic pollution of groundwater, which is largely by arsenite. The bacterium WB3 is also capable of reducing dissolved Fe(III) citrate, solid Fe(III)-oxyhydroxide, and elemental sulfur, using acetate as the electron donor. It is a member of the *Desulfuromonas* genus and possesses a dissimilatory arsenate reductase that was identified using degenerate polymerase chain reaction primers. The sediment from which WB3 was isolated was brown, Pleistocene sand at a depth of 35.2 m below ground level (mbgl). This level was some 3 cm below the boundary between the brown sands and overlying reduced, gray, Holocene aquifer sands. The color boundary is interpreted to be a reduction front that releases As for resorption downflow, yielding a high load of labile As sorbed to the sediment at a depth of 35.8 mbgl and concentrations of As in groundwater that reach >1000 μg/L.



INTRODUCTION

Concentrations of dissolved arsenic (As) in groundwater of deltaic aquifers of SE Asia often exceed 10 μg/L, the World Health Organization's guideline value for As in drinking water.[1,2] The As hazard is most severe in the shallow aquifers of Bangladesh and West Bengal (the Bengal Basin), typically at depths <50 m. Despite more than a decade of remedial action, in 2009 in Bangladesh alone some 42 million people still used groundwater for domestic supply that contained >10 μg/L of As.[3] Consumers of As-polluted water are at risk of adverse effects on health,[4−6] and 1 death in 18 in Bangladesh may be related to ingestion of As-polluted groundwater.[7] Remediation action since 2009 has, however, seen some reduction to As exposure in many areas.[8]

Shallow groundwaters of the Bengal Basin usually contain mg/L of dissolved iron (Fe) as well as As.[9−12] In groundwater worldwide, the presence of aqueous Fe is due to dissimilatory reduction of sedimentary Fe(III)-oxyhydroxides by indigenous bacteria that couple its reduction to the oxidation of organic matter.[13] Since both As(V) and As(III) strongly sorb to Fe(III)-oxyhydroxides, reductive dissolution of Fe(III)-oxyhydroxide has long been invoked as the cause of As pollution in groundwater in the U.S.A.,[14−16] in the Bengal Basin[9,17] and elsewhere.[8] There is, furthermore, common agreement that the process of As-pollution of anoxic groundwaters is mediated by bacteria. This agreement has prompted studies of microbial populations in aquifers undertaken with a view to identifying the bacteria responsible for As pollution of groundwater: for example, molecular techniques and ex situ sediment incubations have been used to show that dissimilatory As(V) reducers contribute to As mobilization in sediments from California and Nevada;[18] Massachusetts,[19] Cambodia,[20] West Bengal,[21] Utah,[22] Colorado,[23] and Japan.[24]

Despite these studies, and the severity of As pollution in the Bengal Basin, no individual bacterium capable of mobilizing As into groundwater has yet been isolated from its sediments. Here, we report the first isolation of such a bacterium, WB3, from aquifer sediments of the Bengal Basin in West Bengal. Our account first explains, for the nonmicrobiological reader, the mechanisms by which bacteria can cause pollution by As, then describes the environment from which WB3 was isolated.

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Finally, we describe some of the properties of WB3 and its method of isolation.

MICROBIOLOGY OF ARSENIC REDUCTION

Biological reduction of As(V) may occur by two separate mechanisms.[25−27] The first mechanism, dissimilatory reduction, is a form of anaerobic respiration whereby bacteria obtain energy from coupling the reduction of As(V) to the oxidation of either an organic (e.g., acetate) or inorganic (e.g., hydrogen) compound.[18,19,25] The second mechanism, detoxification, occurs when As(V) enters the cell interior (the cytoplasm), is reduced, and is then exported as As(III). This process requires an input of energy by the cell.[26]

The free energy available for growth from dissimilatory reduction of As(V) is −252.6 kJ/mol acetate when coupled to acetate oxidation at pH 7.[28] The reduction is catalyzed by the respiratory As(V) reductase (Arr), an enzyme of the dimethyl sulfoxide (DMSO) reductase family of molybdoenzymes.[29] This enzyme is located outside the cytoplasm either attached to the cytoplasmic membrane (in Gram-positive bacteria)[30] or in the periplasm (in Gram-negative bacteria).[29,31] The Arr is composed of two heterologous subunits. One, ArrA is a catalytic subunit that contains molybdenum at its active site and a 4Fe-4S cluster; the other, ArrB contains four 4Fe−4S clusters.[29,31] Respirers of As(V) are phylogenetically diverse and include members of the phyla *Proteobacteria*, *Firmicutes*, and *Chrysiogenetes*. The original discovery of a dissimilatory As(V)-reducing bacterium[25] led to its implication in the release of As to pore water in lake sediments in the U.S.A.,[19] and it was subsequently shown to increase As release from synthetic minerals.[32]

The mechanism of As detoxification, referred to as the As-resistance (Ars) mechanism, occurs if As enters the cell's interior, i.e., its cytoplasm. The similarity in ionic size and charge between dissolved PO_4 and the oxyanions of As(V) leads to both entering the cell via phosphate-transport pathways. The presence of As in the cell impairs its operation because As(V) uncouples oxidative phosphorylation and As(III) binds sulfhydryl groups of proteins. The As resistance mechanism reduces As(V) to As(III) in the cytoplasm, in contrast to dissimilatory reduction which occurs in the periplasm or cytoplasmic membrane. Reduction of As(V) is catalyzed by the As(V) reductase, ArsC. The As(III) is pumped out of the cell via an efflux pump, ArsB. Both ArsC and ArsB are encoded by the *arsC* and *arsB* genes, respectively. The As-resistance mechanism is found in both prokaryotic domains of life.[33]

EXPERIMENTAL PROCEDURES

Study Site and Sample Collection. The sediment from which WB3 was isolated was recovered at a depth of 35.2 mbgl at the location 88.48837°E, 22.74110°N, in the village of Moyna, West Bengal. Percussion coring into 0.5 m hard-plastic core-tubes was undertaken over a target interval between 27 and 41 mbgl, where previous work in Moyna had proven the existence of an interface between gray and brown sands.[34,35] Percussion coring drives a cleaned core-barrel ahead of the drill-string and recovered sections of sediment that were minimally disturbed and unaffected by drilling fluid. Core barrels were of 50 cm length, with core recovery at around 50%. To compensate for poor recovery, three holes spaced 3 m apart were cored at staggered depths to provide as complete a sequence as possible. Previous coring with this method in layered sands showed layering distorted only in the outermost few mm of the core (McArthur et al. unpublished, 2004).

On recovery, cores were placed in a glovebag that was flushed 5 times with oxygen-free nitrogen and contained oxygen-getters. In the glovebag, about 2 cm of core was removed with a hacksaw from each end of the core barrel to remove sediment exposed to air. The remaining core was capped and sealed with electrical tape. All tools and equipment were repeatedly dosed with surgical spirit to reduce contamination by unwanted microorganisms. The cores were kept at ambient temperature for transport to the U.K., where they were transferred to a COY anaerobic chamber and the ends were further trimmed to ensure anaerobic integrity.

A nest of 5 piezometers (Piezometer EP, Figure S1 of the Supporting Information, SI) was installed at the core site using the local hand-operated reverse-circulation method of drilling. The midpoint of the 1 m screens were placed at depths of 28.5, 31.5, 34.6, 37.6, and 40.7 mbgl. This limited depth range was chosen to span the contact of the gray and brown sands. The nearest pumped domestic well was 30 m distant from the field site; the nearest irrigation well was 390 m distant.

Using a surface vacuum pump, groundwater was sampled from piezometers within hours of well development being completed, again 1 week later, and again two months later. Samples were filtered through 0.45 μm membrane filters, collected in 15 mL plastic tubes, and acidified for later analyses. At the well-head on the third sampling, As was speciated using ion-exchange cartridges (Metalsoft, Piscataway, New Jersey).

Chemical Analyses. To assess the amount of labile As in the sediment, subsamples of ∼1 g were leached with 10 mL of London tap water overnight in the dark in a COY anaerobic chamber with an atmosphere of 20:1 N_2/H_2. After centrifugation, the supernatant was diluted with oxygen-free London tap water, and the aqueous As speciated using ion-exchange resin (Metalsoft, Piscataway, New Jersey). Analysis of As(total) and As(III) was done by inductively coupled plasma mass spectrometry (ICP-MS).

Piezometer waters were analyzed for Cl, NO_3, and SO_4 by ion chromatography; for Ca, Mg, Na, K, Fe, and Mn, by ICP atomic emission spectrometry (ICP-AES) and for As by ICP-MS. The concentration of acetate used in microbial incubations was measured using the acetate colorimetric assay kit (Biovision). The concentration of dissolved Fe(II) in cell cultures was measured using ferrozine.[36]

Enrichment, Isolation, and Growth of WB3. A ∼1 g sample of brown sand from 35.2 mbgl was inoculated into an Anoxic Minimal Medium (AMM)[37] containing 10 mM As(V) and 10 mM acetate in a COY anaerobic chamber. The enrichment was incubated at 28 °C without shaking and after 4 weeks the As(V) had been reduced to As(III). The Hungate technique was used for all subsequent culture manipulations.[38] The enrichment was subcultured eight times, serially diluted and plated onto AMM containing 1.5% (w/v) purified agar (Oxoid, Hampshire, U.K.) in the anaerobic chamber. Single colonies were streaked onto the same solid medium then picked and tested for their ability to reduce As(V).

Growth experiments were performed in 50 mL AMM in 100 mL serum bottles in triplicate, from independent starter cultures. Potential electron acceptors were added at concentrations of 10 mM in 10 mL AMM, except elemental sulfur which was added as a solid in an anaerobic chamber. At regular time intervals, aliquots were taken for cell counting and

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centrifuged and frozen for chemical analyses. Growth was determined by increase in cell number after 5 days.

Molecular Biology and Phylogenetic Analyses. DNA was isolated from WB3 cells using the Powersoil DNA isolation kit (MoBio, Calrsbad, California) according to the manufacturer's instructions. The almost complete 16S rRNA gene of WB3 was amplified and sequenced as described previously with 27f and 1525r primers.[39,40] A portion of the *arrA* gene was amplified by PCR with BioTaq (Bioline, London, U.K.) DNA polymerase and the primers TOArrAF (GGATAAAAACACCGATGAAGMGNACNAAYC) and TOArrAR (CGTTGGCTCTTTCCCASWYTCNGGRT), which were designed with the iCODEHOP program[41] using all available complete ArrA sequences from GenBank available at the time (March 2012). PCR reactions contained final concentrations of 4 ng/μL DNA and 1 ng/μL forward and reverse primers. The PCR conditions were as follows: 95 °C for 2 min followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1.5 min with a final extension of 5 min at 72 °C.

Attempts to amplify a portion of *arrA* and *arsC* with published primer sets were performed as previously described.[42−44] PCR products were cloned into pGEMt vector (Promega) according to the manufacturer's instructions and both strands sequenced at the Wolfson Institute of Biomedical Research, University College London. Sequencing was performed by capillary electrophoresis with a 3730XL Genetic Analyzer and BigDye 3.1 (Applied Biosystems, Paisley, U.K.).

Nucleotide and deduced protein sequences were aligned with MUSCLE[45] and neighbor-joining trees constructed using MEGA 5.05.[46] The Kimura-2-parameter method and Jones-Taylor-Thornton computations were used for nucleotide and protein trees, respectively. The 16S rRNA gene and *arrA* sequences were deposited in GenBank with the accession numbers KM452745 and KM452746, respectively.

RESULTS

Sedimentology. The sediments at the core site were gray, reduced, Holocene sands to around 35 m depth, overlying brown, late Pleistocene sands from 35 mbgl to 47 mbgl, in keeping with a similar stratigraphy found elsewhere in much of Moyna.[34,35] The interface between gray and brown sand was at 35.2 mbgl and captured in two cores (SI Figure S2). The interface was sharp, occurring over millimeters of core. The contact lacked sedimentological expression, such as a change in sediment grain size; both gray and brown sands were fine-to-medium grade sand and so the color change is interpreted to be a redox interface.

Hydrochemistry. The composition of groundwater from piezometer EP is given in SI Table S1. In and around Moyna, concentrations of As and Fe in groundwater from the upper gray sands are ≤1180 μg/L As and ≤13.7 mg/L Fe, while groundwater from the lower brown sands contain typically <10 μg/L As and <1 mg/L Fe.[34] Samples of groundwater from Piezometer EP reflect this distribution (Figure 1). Groundwaters from the upper two piezometers (28.5 and 31.5 mbgl) contain high levels of Fe and As (5−6 mg/L Fe, 150−160 μg/L As); the As is >97% As(III) which is consistent with previous data from Moyna (SI Table S2). Groundwaters from the lower two piezometers (37.6 and 40.7 mbgl) contain little Fe or As (<200 μg/L Fe, < 9 μg/L As), and the concentrations of As were too low for accurate speciation. In groundwater from the middle piezometer (34.6 mbgl), the concentration of Fe was 0.05 mg/L and that of As was 1018 μg/L, 87% of which was

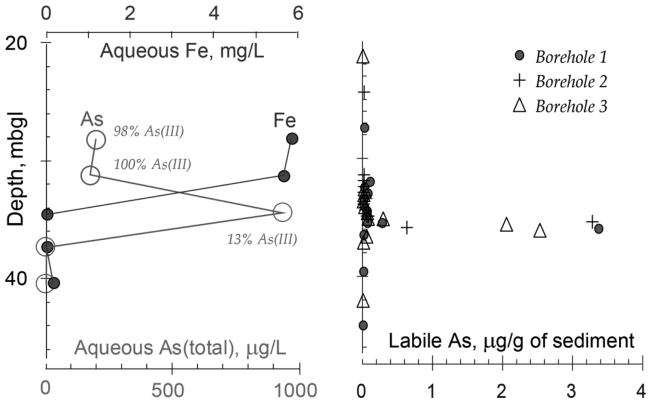

Figure 1. Vertical profiles of As (Total) and Fe in (a) piezometers; points are joined by lines to emphasize trends, while accepting that this may alias the true detail of the profile. (b) Leaches of cored sediment with deionized water (leaches with phosphate solutions gave similar results).

As(V). This peak of dissolved As was reflected in the sediments content of water-leachable As (Figure 1b, SI Table S3), which showed a maximum of 3.4 μg/g sediment between 35.4 and 36.1 mbgl.

Isolation and Growth of WB3. To date, no As(V)-respiring organism has been isolated from Bengal delta sediments, so the objective of this study was to search for such an organism at the redox front where we expect As to be released and its reduction to be occurring. Enrichments were done in a minimal medium with As(V) as the terminal electron acceptor and acetate as the electron donor. An organism, here designated WB3, was isolated and found to be a strictly anaerobic rod-shaped, motile bacterium. Growth of WB3 with 5 mM (soluble) As(V) and 5 mM acetate is shown in Figure 2.

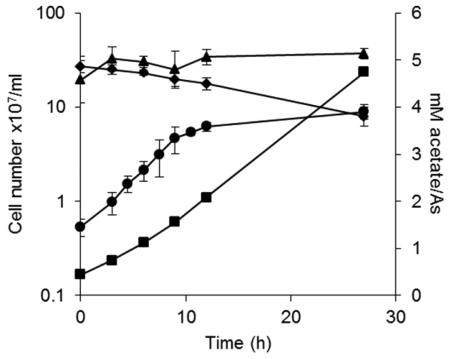

Figure 2. Growth curve of WB3 grown with As(V) as the terminal electron acceptor and acetate as the electron donor. Formation of As(III) and total As are shown. Symbols and error bars represent the mean and standard deviation of three independent experiments. ●, cell number; ■, As(III); ♦, acetate; and ▲, As(total).

The stoichiometry of As(V) reduction was 4.05 mol As(V) to 1 mol acetate oxidized, consistent with dissimlatory As(V) reduction.[28] The generation time for WB3 was 2.9 (±0.13) h. No growth was observed in the absence of acetate or As(V), or when the culture was exposed to air. The majority of the As(V) was reduced during the postexponential phase of growth as this is where the cell density was at its maximum. With acetate as the electron donor, WB3 was also able to use soluble Fe(III) citrate, amorphous Fe(III)-oxyhydroxide, and elemental sulfur as electron acceptors but could not use As(III), nitrate, or

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nitrite. When both Fe(III) citrate (5 mM) and soluble As(V) (2.5 mM) were provided as electron acceptors, they were reduced concomitantly.

Identification of WB3. The almost complete 16S rRNA gene of WB3 was sequenced and found to be most closely related to members of the *Desulfuromonas* and *Pelobacter* genera in the *Desulfuromonadaceae* family of the Deltaproteobacteria. Phylogenetic analysis of published *Pelobacter* and *Desulfuromonas* species (Figure 3) revealed that WB3 is most

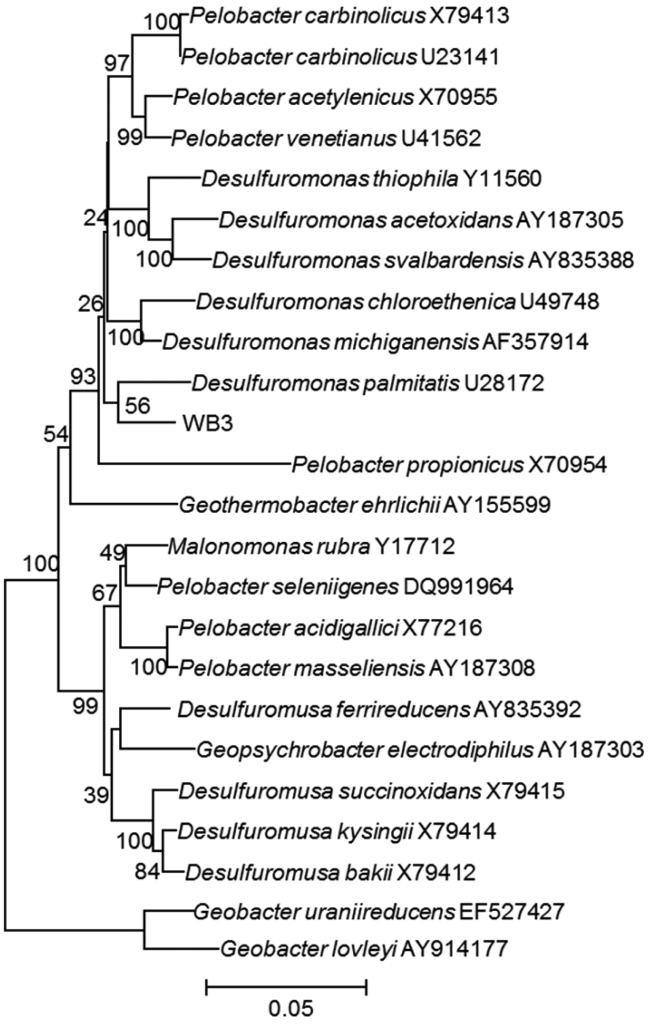

Figure 3. Phylogenetic tree of 16S rRNA gene sequences from the *Desulfuromonadaceae* with strain WB3 and representatives of the *Geobacter* genus. The tree was rooted with *Methanocaldococcus jannaschii* (L77117) (not shown) and significant bootstrap values are shown (per 100 retrials). Accession numbers are shown.

closely related to *Desulfuromonas palmitatis* (95% sequence identity) an Fe(III)-reducing bacterium isolated from hydrocarbon-contaminated sediment in San Diego Bay, U.S.A.[47]

Mechanism of As(V) Reduction. To understand the role of WB3 in the mobilization of As in West Bengal, it was essential to confirm the mechanism(s) of As(V) reduction. Attempts to amplify portions of the *arrA* gene using previously designed degenerate primers[43,44] were unsuccessful. New primers were designed and were used to amplify 58% (1502 bp) of the *arrA* gene by PCR. Identification of the *arrA* gene in WB3 indicates that respiratory As(V) reduction in this organism is catalyzed by Arr. When compared to sequences from isolates, the partial ArrA sequence was most closely related to those from *Geobacter uraniireducens* and *Geobacter lovleyi* (72% and 70% sequence identity, respectively) (Figure 4), which are also members of the *Desulfuromonodaceae* family.

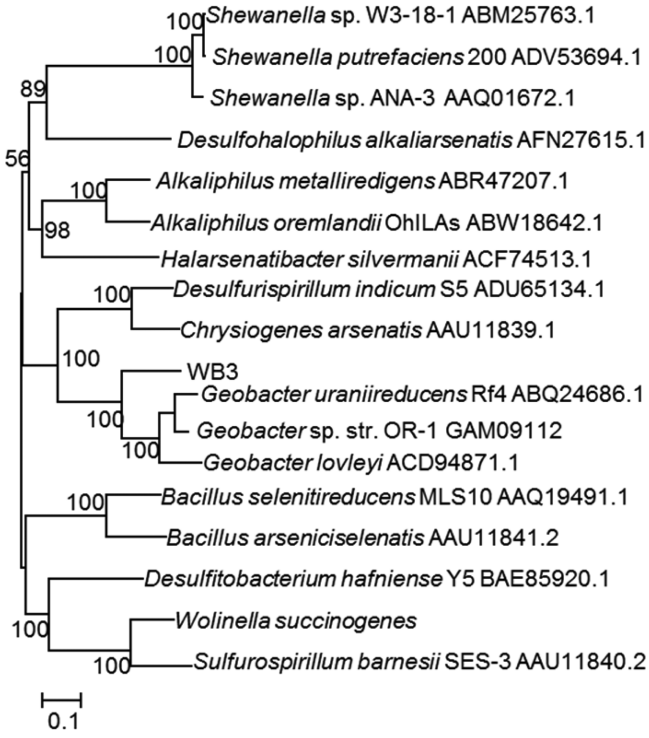

Figure 4. Phylogenetic tree of known ArrA protein sequences with WB3. Tree was rooted with the AioA from NT-26 (AAR05656.1) (not shown). Significant bootstrap values (per 100 retrials) and accession numbers are shown.

When compared to sequences from environmental samples, the WB3 ArrA shares 80% sequence identity with partial sequences identified in microcosms of Utah basin sediment, where As(V) was released to the soluble phase,[22] and in As-contaminated glauconite sediments, New Jersey.[48]

Attempts were made to identify the As-resistance gene, *arsC* however no homologues were detected using previously designed primers.[42] Our inability to detect *arsC* does not mean it is absent, as it may have escaped detection owing to the great sequence diversity among *arsC* genes.

DISCUSSION

Hydrochemistry. In Moyna, measured concentrations of As ands Fe in groundwater from the upper gray sands are ≤1180 μg/L As and ≤13.7 mg/L Fe, while groundwater from the lower brown sands contain typically <10 μg/L As and <1 mg/L Fe.[34,35] The composition of groundwater from Piezometer EP reflects this distribution (Figure 1 and SI Figure S3). In the upper gray sands, groundwater has thus reached or passed the stage of Fe(III)-reduction. In the underlying brown sands, groundwater is poised at Mn(II)-reduction.[49]

The interface of the gray and brown sands lacked sedimentological expression, such as a coarsening downward sequence, or a change in grain size. It is therefore interpreted as a redox interface at which underlying brown sands are being reduced by downward movement of Fe(II)-rich, reducing,

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groundwater from shallower levels. We collected two cores in which this boundary occurred (SI Figure S2) at a depth of 35.2 mbgl. In Moyna, the minimum measured downward rate of groundwater movement across the hydrodynamically unrestricted interface between gray sand and brown sand was 0.05 m/d at Piezometer FP[35] (location on SI Figure S1). Rates at Piezometer EP are likely to be below 0.05 m/d because of the dearth of nearby pumping wells compared to Piezometers AP and FP.

The interpretation of the brown/gray interface as a redox boundary is consistent with the fact that Fe concentrations in piezometer water decrease from 6 mg/L at 31.5 to 0.2 mg/L at 34.6 mbgl (Figure 1; SI Figure S3; Table S1). Dissolved Fe, as Fe(II), is presumably removed from downward-moving groundwater by reaction with sedimentary Fe(III)-oxyhydroxide in the brown sands. The slight offset of depth between the As maxima in the piezometers and the As maxima in labile As in the sediments is likely due to small uncertainties in the depths estimated by the drilling methods used to core and that used to install piezometers.

The profile of labile As (Figure 1b) shows that, 60 cm below the redox front, at 35.8 mbgl, a maximum occurs in labile As, which we interpret to be a front where As is sorbed to brown sand from downward-moving groundwater. At this front, sorbing As will derive both from aqueous As in the As-rich groundwater from the upper aquifer of gray sand, and As released (if any) from the redox front just above the sorption front. The near coincidence close to the interface of the gray and brown sands of maxima in both dissolved and sorbed As, and the fact that As(V) comprises 87% of dissolved As (Figure 1, SI Figure S3) at 34.6 mbgl, suggests that As is released from Fe(III)-oxyhydroxide as As(V) prior to its reduction.

Our data may provide an explanation for the previous observation[50] that of 3500 wells in Bangladesh tested for As,[10,11] 6% contained both <1 mg/L Fe and >50 μg/L of As, suggesting to those authors that "Fe dissolution is not always required for significant As release". Such groundwaters may be from wells in which the screens, which are typically 12 feet long, span sorption fronts from which sorbed As can be flushed by the well's disturbance of groundwater flow during pumping. Other occasional reports occur of extreme concentrations of As that are accompanied by low concentrations of Fe may have the same explanation; examples of such wells include Ba 232 in Moyna, containing 1130 μg/L As and 0.7 mg/L Fe[34] and, possibly, wells TM-1 and TM-2 in Beldanga, Murshidabad, West Bengal, with As concentrations of 4400 and 4600 μg/L,[51] although these authors report no Fe data.

Our interpretation is consistent with peak aqueous As found in two other piezometers in Moyna (AP and FP;[35] SI Figure S4) which show peak concentrations at deeper levels below the interface of brown and gray sand than is found at EP. Compared to EP, the higher density and closer proximity of wells around AP and FP has resulted in more draw-down of the redox front and so the As peak occurs at deeper levels than at EP. In all cases where such peak concentrations are seen in piezometers, we acknowledge that the real shape of the profile may have been distorted (aliased) by the low sampling resolution; higher resolution sampling is needed to reveal the true composition of the groundwater gradients.

To our knowledge, this study is the smallest scale on which the redox fronts in the Bengal Basin have been observed, and should warn against the positioning of well screens close to redox boundaries because of their associated sorption fronts where As is so labile.

Microbiology. Phylogenetically diverse organisms capable of oxidizing As(III) and resisting As have been isolated from samples from the Bengal Basin.[52,53] Organisms capable of both As(V) and Fe(III) reduction have been isolated from Japanese soil (e.g., *Anaeromyxobacter* sp. str. PSR-1 and *Geobacter* sp. str. OR-1) and shown to cause As release in both microcosm experiments and cultures with synthetic minerals.[24,54] A similar metabolic capability may drive As release in the Bengal Basin and elsewhere but so far such an organism has not been isolated. *Geobacter* 16S rRNA genes and *arrA* gene sequences have been identified in microcosms of West Bengal sediments, where As(V) was released to the soluble phase.[21] Here we report the first isolation and identification of a dissimilatory As(V) reducer, WB3, from a redox front in aquifer sediments from the Bengal delta. The characterization of these types of organisms is critical to understanding As-pollution. WB3 is the first member of the *Desulfuromonadaceae* family that can respire with As(V). Like other members of the genus *Desulfuromonas*,[55] it can use elemental sulfur, soluble Fe-citrate and amorphous Fe(III)-oxyhydroxide as terminal electron-acceptors. The ability to respire both As(V) and Fe(III) means that it could play a role in the mobilization of As and Fe into groundwater in the Bengal delta. Its role in As mobilization remains to be explored but we hypothesize that the As(V) is reduced to As(III) when in solution and not when bound to Fe. We do so for two reasons. First, dissolved As at the sorption front comprises 87% As(V) which, given the anoxic condition of the groundwater, can only mean that it was released as As(V) and is undergoing reduction: dissolved As(III) is the predominant form of As in groundwater of the Bengal Basin (Figure 1, SI Figure S2, Table S2). Second, a homologue of the *arrA* gene has been identified in WB3 (not been previously identified in the genera, *Pelobacter* or *Desulfuromonas*). WB3 is a Gram-negative bacterium and like other Gram-negative dissimilatory arsenate respirers, the Arr in these organisms is located in the periplasm, a location that prevents it reducing As(V) bound to solid Fe(III)-oxyhydroxide. We therefore propose that As pollution in the Bengal basin is caused by the reduction of Fe(III)-oxyhydroxide, releasing sorbed As(V) to the soluble phase, which is subsequently reduced by organisms like WB3. It is logical to assume that similar conditions exist in other aquifers worldwide where groundwater contains both As and Fe at unacceptable concentrations.

Other Microbial Mechanisms of As Mobilization. Space does not permit a thorough review of previous examinations of microbial mobilization of As, but a few comments are worthwhile as a conclusion to this study.

The fact that leaching sediment with water can mobilize As[56] might suggest that reductive dissolution alone may not explain As pollution. Such a finding, however, is consistent with re-equilibration into As-free water of As sorbed onto sediment from As-rich groundwater.

The hypothesis of reductive dissolution of Fe(III)-oxyhydroxide as a mechanism of As-pollution has been questioned by the observation that of groundwaters from As-polluted regions of the Bengal Basin 6% contain <1 mg/L of Fe and more than 50 μg/L As.[50] This leaves open the reason as to why 94% do not.

Further objections arise from the use of microbial microcosms that show release of As to solution can apparently occur independently of the release of Fe(II) to solution.[19,57] Such

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findings contrast with others that show concomitant microbial release from solids to solution of both Fe(II) and As.[18,21,58,59] Almost all such studies have used concentrations of reactants that are higher than those encountered in aquifers, or organisms that have not been shown to be present in aquifers, or substrates that have not been shown to host As in aquifers (e.g., iron arsenate). While such studies may be relevant to As release to groundwater, that relevance has not yet been demonstrated. Moreover, any microbial experiments that use sediment from an As-polluted aquifer as the reactant, seldom consider the history of that sediment (which is uncertain). If the sediment used derives from a shallow palaeo-channel aquifer in the Bengal Basin, for example, then it is conceivable, even likely, that it has been through a process of microbial mobilization of As. The consequence of using such material for microcosm studies is that the *product* of reactions that mobilize As is then used as a *reactant* in studies designed to elucidate reactions that mobilize As. The results of such a procedure may not provide the information sought.

■ ASSOCIATED CONTENT

Supporting Information

Further information on the field locations, piezometer profiles, sediment cores, and leaching experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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